Remarks

The title has been amended to more precisely reflect the presently claimed invention. The specification has been amended to add priority information and headings, to correct the name and address of the depository, and to correct the references to the Figures such that they conform with the Figures submitted concurrently herewith. The Sequence Listing has been added to bring the present application in compliance with 37 C.F.R. § 1.821. A Request Under 37 C.F.R. § 1.821(e) is filed concurrently herewith. No new matter has been added.

Respectfully submitted,

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Jonathan L. Klein Reg. No. 41,119
Attorney for Applicants

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20850
Telephone: (301) 251-6015

JLK/SA/ba

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Yu et al.
Application Serial No.: Unassigned
Filed: Herewith
Title: Colon Specific Genes and Proteins

Attorney Docket No.: PF160D2
Art Unit: Unassigned
Examiner: Unassigned

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Text has been amended as indicated by brackets [ ] (for deletions) and underlining (for additions).

In the specification:

Page 5, seventh full paragraph, which begins "Figure 1 is...":

Figure 1 is a partial cDNA sequence SEQ ID NO:1 and the corresponding deduced amino acid sequence SEQ ID NO:2 of a colon specific gene of the present invention.

Page 5, eighth full paragraph, which begins "Figure 2 is...":

[Figure 2 is] Figs. 2A and 2B, collectively and consecutively, show a partial cDNA sequence SEQ ID NO:3 and the corresponding deduced amino acid sequence SEQ ID NO:4 of a colon specific gene of the present invention.

Page 5, ninth full paragraph, which begins "Figure 3 is...":

[Figure 3 is] Figs. 3A and 3B, collectively and consecutively, show a partial cDNA sequence SEQ ID NO:5 of a colon specific gene of the present invention.

Page 6, first full paragraph, which begins "Figure 4 is...":

Figure 4 is a partial cDNA sequence SEQ ID NO:6 and the corresponding deduced amino acid sequence SEQ ID NO:7 of a colon specific gene of the present invention.

Page 6, second full paragraph, which begins "Figure 5 is...":

[Figure 5 is] Figs. 5A and 5B, collectively and consecutively, show a partial cDNA sequence SEQ ID NO:8 and the corresponding deduced amino acid sequence SEQ ID NO:9 of a colon specific gene of the present invention.

Page 6, third full paragraph, which begins "Figure 6 is...":

Figure 6 is a partial cDNA sequence SEQ ID NO:10 and the corresponding deduced amino acid sequence SEQ ID NO:11 of a colon specific gene of the present invention.

Page 6, fourth full paragraph, which begins "Figure 7 is...":

Figure 7 is a partial cDNA sequence SEQ ID NO:12 of a colon specific gene of the present invention.

Page 6, fifth full paragraph, which begins "Figure 8 is...":

Figure 8 is a full length cDNA sequence SEQ ID NO:13 and the corresponding deduced amino acid sequence SEQ ID NO:14 of a colon specific gene of the present invention.

Page 6, sixth full paragraph, which begins "Figure 9 is...":

[Figure 9 is] Figs. 9A and 9B, collectively and consecutively, show a full length cDNA sequence SEQ ID NO:15 and corresponding deduced amino acid sequence SEQ ID NO:16 of [a] the CSG10 colon specific gene of the present invention.

Page 6, seventh full paragraph, which begins "Figure 10 is...":

Figure 10 is a partial cDNA sequence SEQ ID NO:17 and corresponding deduced amino acid sequence SEQ ID NO:18 of a colon specific gene of the present invention.

Page 6, eighth full paragraph, which begins "Figure 11 is...":

Figure 11 is a partial cDNA sequence SEQ ID NO:19 and the corresponding deduced amino acid sequence SEQ ID NO:20 of a colon specific gene of the present invention.

Page 6, ninth full paragraph, which begins "Figure 12 is...":

Figure 12 is a partial cDNA sequence SEQ ID NO:21 of a colon specific gene of the present invention.

Page 6, tenth full paragraph, which begins "Figure 13 is…":

Figure 13 is a partial cDNA sequence SEQ ID NO:22 of a colon specific gene of the present invention.

Page 6, twelfth full paragraph extending onto page 7, which begins "In accordance with one aspect…":

In accordance with one aspect of the present invention there is provided a polynucleotide which encodes one of the mature polypeptides having the deduced amino acid sequence of [Figure 8 or 9] Fig. 8 or of Figs. 9A and 9B collectively, and fragments, analogues and derivatives thereof.

At page 7, first full paragraph, which begins "In accordance with a further aspect…":

In accordance with a further aspect of the present invention there is provided a polynucleotide which encodes the same mature polypeptide as a human gene having a coding portion which contains a polynucleotide which is at least 90% identical (preferably at least 95% identical and most preferably at least 97% or 100% identical) to one of the polynucleotides of [Figures 1-7 or 9-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-7, 9A-9B and 10-13, as well as fragments thereof.

Page 7, third full paragraph, which begins "In accordance with yet another aspect…", and replace therewith the following paragraph:

In accordance with yet another aspect of the present invention, there is provided a polynucleotide probe which hybridizes to mRNA (or the corresponding cDNA) which is transcribed from the coding portion of a human gene which coding portion includes a DNA sequence which is at least 90% identical to (preferably at least 95% identical to) and most preferably at least 97% or 100% identical) to one of the polynucleotide sequences of [Figures 1-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-8, 9A-9B and 10-13.

Page 7, fourth full paragraph, which begins "The present invention further relates...":

The present invention further relates to a mature polypeptide encoded by a coding portion of a human gene which coding portion include a DNA sequence which is at lest 90% identical to (preferably at least 95% identical to and more preferably 97% or 100% identical to) one of the polynucleotides of [Figures 1-7 or 10-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-7, and 10-13, as well as analogues, derivatives and fragments of such polypeptides.

Page 8, first full paragraph, which begins "The present invention also relates...":

The present invention also relates to one of the mature polypeptides of [Figures 8 or 9] Fig. 8 or of Figs. 9A and 9B, collectively, and fragments, analogues and derivatives of such polypeptides.

Page 8, third full paragraph, which begins "In accordance with an aspect...":

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode for the mature polypeptides having the deduced amino acid sequence of [Figures 8 or 9] Fig. 8 or of Figs. 9A and 9B, collectively, collectively, or fragments, analogues or derivatives thereof.

Page 8, fourth full paragraph, which begins The polynucleotides of the present invention...":

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may include DNA identical to [Figures 1-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-8, 9A-9B and 10-13 or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the coding sequence of a gene

which coding sequence includes the DNA of [Figures 1-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-8, 9A-9B and 10-13 or the deposited cDNA.

Page 9, fourth full paragraph, which begins "The polynucleotides of the invention...":

The polynucleotides of the invention may have a coding sequence which is a naturally occurring allelic variant of the human gene whose coding sequence includes DNA as shown in [Figures 1-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-8, 9A-9B and 10-13 or of the coding sequence of the DNA in the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

Page 10, third full paragraph extending onto page 10, which begins "The present invention further relates...":

The present invention further relates to polynucleotides which hybridize to the hereinabove-described polynucleotides if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide of the present invention encoded by a coding sequence which includes the DNA of [Figures 1-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-8, 9A-9B and 10-13 or the deposited cDNA(s).

Page 11, second full paragraph, which begins "Thus, the present invention is...":

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% and more preferably at least 95% identity to a polynucleotide which encodes the mature polypeptide encoded by a human gene which

includes the DNA of one of [Figures 1-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-8, 9A-9B and 10-13 as well as fragments thereof, which fragments have at least 30 bases and preferably at least 50 bases and to polypeptides encoded by such polynucleotides.

At page 11, please delete the fourth full paragraph extending onto page 12, which begins "The partial sequences of Figures 1-7...", and replace therewith the following paragraph:

The partial sequences of [Figures 1-7 and 10-13] Figs. 1, 2A-2B, 3A-3B, 4, 5A-5B, 6-7 and 10-13 may be used to identify the corresponding full length gene from which they were derived. The partial sequences can be nick-translated or end-labelled with ^{32}P using polynucleotide kinase using labelling methods known to those with skill in the art (Basic Methods in Molecular Biology, L.G. Davis, M.D. Dibner, and J.F. Battey, ed., Elsevier Press, NY, 1986). A lambda library prepared from human colon tissue can be directly screened with the labelled sequences of interest or the library can be converted en masse to pBluescript (Stratagene Cloning Systems, La Jolla, CA 92037) to facilitate bacterial colony screening. Regarding pBluescript, see Sambrook et al., Molecular Cloning-A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), pg. 1.20. Both methods are well known in the art. Briefly, filters with bacterial colonies containing the library in pBluescript or bacterial lawns containing lambda plaques are denatured and the DNA is fixed to the filters. The filters are hybridized with the labelled probe using hybridization conditions described by Davis et al., supra. The partial sequences, cloned into lambda or pBluescript, can be used as positive controls to assess background binding and to adjust the hybridization and washing stringencies necessary for accurate clone identification. The resulting autoradiograms are compared to duplicate plates of colonies or plaques; each exposed spot corresponds to a positive colony or plaque. The colonies or plaques are selected, expanded and the DNA is isolated from the colonies for further analysis and sequencing.

At page 21, third full paragraph, which begins "The polypeptides of the present invention...", and replace therewith the following paragraph:

The polypeptides of the present invention include the polypeptides of [Figures 8 and 9] Fig. 8 and of Figs. 9A and 9B, collectively, (in particular the mature polypeptides)

as well as polypeptides which have at least 70% similarity (preferably at least a 70% identity) to the polypeptides of [Figures 8 and 9] Fig. 8 and of Figs. 9A and 9B, collectively, and more preferably at least a 90% similarity (more preferably at least a 90% identity) to the polypeptides of [Figures 8 and 9] Fig. 8 and of Figs. 9A and 9B, collectively, and still more preferably at least a 95% similarity (still more preferably at least 95% identity) to the polypeptides of [Figures 8 and 9] Fig. 8 and of Figs. 9A and 9B, collectively, and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

Page 43, second full paragraph extending onto pages 44 and 45, which begins "The DNA sequence encoding a polypeptide...":

The DNA sequence encoding a polypeptide of the present invention, ATCC # [97201] 97102, which one is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed protein (minus the signal peptide sequence) and the vector sequences 3' to the gene. Additional nucleotides corresponding to the DNA sequence are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer may contain, for example, a restriction enzyme site followed by nucleotides of coding sequence starting from the presumed terminal amino acid of the processed protein. The 3' sequence may, for example, contain complementary sequences to a restriction enzyme site and also be followed by nucleotides of the nucleic acid sequence encoding the protein of interest. The restriction enzyme sites correspond to the restriction enzyme sites on a bacterial expression vector, for example, pQE-9 (Qiagen, Inc. Chatsworth, CA). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with the restriction enzymes corresponding to restriction enzyme sites contained in he primer sequences. The amplified sequences are ligated into pQE-9 and inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform an E. coli strain, for example, M15/rep 4 (Qiagen) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are

identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.^{600}) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized protein is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). The protein is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein is dialyzed to 10 mmolar sodium phosphate.

Page 47, first full paragraph, which begins "Preparation of templates by PCR_...":

Preparation of templates by PCR is a modification of the method of Rosenthal et al. (Rosenthal, et al., Nucleic Acids Res., 1993, 21:173-174). Colonies containing cDNA cloned into pBluescript II or rescued pBluescript phagemid are grown overnight in LB containing ampicillin in a 96 well tissue culture plate. Two µl of the cultures are used as template in a PCR reaction (Saiki, RK, et al., Science, 239:487-493, 1988; and Saiki, RK, et al., Science, 230:1350-1354, 1985) using a tricine buffer system (Ponce and Micol., Nucleic Acids Res., 1992, 20:1992.) and 200 µM dNTPs. The primer set chosen for amplification of the templates is outside of primer sites chosen for sequencing of the templates. The primers used are 5'-ATGCTTCCGGCTCGTATG-3' (SEQ ID NO:23) which is 5' of the M13 reverse sequence in pBluescript and 5'-GGGTTTTCCCAGTCACGAC-3' (SEQ ID NO:24), which is 3' of the M13 forward primer in pBluescript. Any primers which correspond to the sequence flanking the M13

forward and reverse sequences can be used. Perkin-Elmer 9600 thermocyclers are used for amplification of the templates with the following cycler conditions: 5 min at 94°C (1 cycle); (20 sec at 94°C); 20 sec at 55°C (1 min at 72°C) (30 cycles); 7 min at 72°C (1 cycle). Following amplification the PCR templates are precipitated using PEG/NaC1 and washed three times with 70% ethanol. The templates are resuspended in water.